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ORIGINAL ARTICLE

2,4,6-Trinitrotoluene (TNT) air concentrations, hemoglobin changes, and anemia cases in respirator protected TNT munitions demilitarization workers

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Abstract
Purpose 2,4,6-Trinitrotoluene (TNT) is an explosive used in munitions production that is known to cause both aplastic and hemolytic anemia in exposed workers. Anemia in a TNT worker is considered a sentinel health event (occupational) (SHE(O)) in the United States (US). Deaths have been reported secondary to aplastic anemia. Studies have shown that TNT systemic absorption is significant by both the respiratory and dermal routes. No studies encountered looked at hemoglobin change or anemia cases in respiratory protected workers. It is hypothesized that respiratory protection is insufficient to protect TNT workers from the risk of anemia development and hemoglobin concentration drop.
Methods A records review of eight groups of respiratory protected TNT workers' pre-exposure hemoglobin levels were compared with their during-exposure hemoglobin levels for statistically significant (alpha level 0.05) hemoglobin level changes, and anemia cases were recorded. A curve estimation analysis was performed between mean TNT air concentrations and mean hemoglobin change values.
Results Statistically significant hemoglobin level drops and anemia cases were apparent at TNT air concentrations about the REL and PEL in respiratory protected workers. There were no anemia cases or statistically significant hemoglobin level drops at concentrations about the TLV, however. A statistically significant inverse non-linear regression model was found to be the best fit for regressing hemoglobin change on TNT air concentration.
Conclusions Respiratory protection may be inadequate to prevent workers who are at risk for TNT skin absorption from developing anemia. This study contributes evidence that the TLV should be considered for adoption as the new PEL.

Keywords Reticulocytosis · 4-Aminodinitrotoluene · Aplastic anemia · Breathing zone · Time weighted average

The stated views are those of the author and do not represent the views or the policy of the Department of the Army or the Department of Defense of the United States of America.

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Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
APF	Assigned protection factor
ASCAD	Atherosclerotic coronary artery disease
ASPVD	Atherosclerotic peripheral vascular disease
BEI	Biological exposure indices
BMI	Body mass index
BZ	Breathing zone
CBC	Complete blood count
CFR	Code of Federal Regulations (USA)
CHF	Congestive heart failure
COPD	Chronic obstructive pulmonary disease
GA	General area

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Hgb	Hemoglobin
LDH	Lactate dehydrogenase
LFT	Liver function test
MAC	Maximum allowable concentration
NIOSH	National Institute of Occupational Safety and health
OSHA	Occupational Safety and Health administration
PBX	Plastic bonded explosive
PEL	Permissible exposure limit
PPE	Personal protective equipment
QA	Quality assurance
RBCs	Red blood cells
REL	Recommended exposure limit
SES	Socioeconomic status
SHE(O)	Sentinel health event (occupational)
SOP	Standing operating procedure
TLV	Threshold limit value
TNT	Trinitrotoluene
TWA	Time weighted average
UA	Urinalysis

Introduction

2,4,6-Trinitrotoluene (TNT) is a nitro-aromatic explosive compound used in the manufacture of munitions. Originally developed as a yellow dye in Germany by Joseph Wilbrand in 1863, it's use as an explosive in munitions production was only appreciated in 1902 when Germany began loading artillery shells with it. Since this time, TNT has become the most widely used military explosive and is the 'standard' high explosive (HE) against which all other high explosives have been, and continue to be, measured (DA Technical Manual (DA TM) 9-1300-214 1984; Wikipedia 2008). TNT, the first high explosive, has a detonation velocity greater than 22,000 feet per second. It is considered an insensitive explosive; insensitive explosives are characterized by their resistance to detonation by impact or friction. These properties, unlike nitroglycerin for example, allow TNT to be handled by workers in a relatively safe manner. TNT will burn in the presence of oxygen but will explode in an oxygen-deficient environment (such as a bomb body casing). Intrinsically safe capital goods are needed in plants that handle TNT to prevent fugitive electricity, static electricity, or certain radiofrequencies that could lead to detonation. Workers who work with open TNT must be conductive to dissipate any potential static electricity build-up and this is best accomplished by the wear of conductive footwear, cotton coveralls and caps (DA TM 0-1300-214; Bodeau 1993). Additional physical characteristics of TNT are delineated elsewhere (2,4,6-TNT MSDS, Zaklady Chemiczne, Wojska, Polskiego 2003; 2,4,6-TNT, NIOSH Pocket Guide to Chemical Hazards 2005).

TNT is not only a munitions worker safety hazard due to detonation and flammability potential but is also a health hazard with a track record of deaths secondary to aplastic anemia and hepatic necrosis. Some historical numbers are sobering: In the United States, from 1914 to 1918, there were 24,000 recorded cases of TNT poisoning, of which 580 ended fatally, and in the United Kingdom, from 1916 to 1941, there were 465 cases of poisonings, of which 125 were fatal (Bodeau 1993). Indeed, anemia has been a traditional problem in the TNT explosive worker population and has been supported by epidemiological studies as well as animal modeling (ACGIH 2001; Army 1976; Bodeau 1993; Crawford 1954; Djerassi and Vitany 1975; Hathaway 1977; Hunter 1978; LaDou 2007; Opresko 2004; Richter-Torres et al. 1995; Sabbioni et al. 2005). Anemia in worker(s) working with TNT is considered an Occupational Sentinel Health Event (SHE(O)) (Mullan and Murthy 1991). Although much is still unknown as to the mechanisms causing aplastic anemia, there is evidence, at least as far as the erythropoetic line is concerned, that TNT and/or a toxic metabolite suppresses delta-aminolevulinic acid synthase and heme synthase leading to early marrow hyperplasia followed by hypoplasia (Bodeau 1993; Richter-Torres et al. 1995). TNT's mechanism for causing hemolytic anemia is a bit better understood and is known to involve oxidative damage to red blood cells (RBC) via the production of methemoglobin. The hemolysis is predominantly extravascular and involves splenic sequestration of damaged RBCs (Bodeau 1993; Hunter 1978; LaDou 2007). It has been postulated that TNT hydroxyarylamine metabolites are responsible for hemoglobin oxidation (Liu et al. 1992), as well as for sulfinamide adducts with hemoglobin cysteine residues (Sabbioni et al. 2005).

Some specific examples from the literature concerning TNT-induced anemia, hemoglobin drop, and anemia-related deaths are as follows: Case series reports of TNT-induced aplastic anemia deaths (Cone 1944; Crawford 1954; Eddy 1944; Hart and Ley 1944; Hathaway 1977; Hunter 1978; McConnell and Flinn 1946; Voegtlin et al. 1921). Dacre and Rosenblatt (1974) showed no species difference with regard to anemia and hemolysis after oral feedings of TNT to cats, dogs, rabbits, and rats. Cone (1944) described the effects of TNT on formed elements of the blood, in particular RBC destruction. Hathaway (1977) suggests difficulty in formulating dose–response relationships for airborne levels of TNT and observed toxic effects in man due to skin absorption problems and accidental ingestions. Animal anemia dose response to oral TNT dosing in rats and dogs was shown by Dilley and Tyson (1982), Levine et al. (1984), and Levine et al. (1990). TNT

induced hemolytic anemia deaths in 3 TNT workers with Glucose-6-phosphate dehydrogenase (G6PD) deficiency (Djerassi and Vitany 1975). Gribova et al. (1983) in the USSR showed erythrocyte numbers and hemoglobin concentration reductions as a function of TNT air concentrations; the higher the TNT air concentrations, the lower the RBC count and hemoglobin concentration. The US Army (1976) did a cross-sectional study of TNT workers versus non-TNT workers at 5 ammunition plants, which revealed a low-grade hemolysis with early compensatory reticulocytosis as a function of lower TNT air concentrations (<0.5 mg/m^3) but progressing to anemia and reduced reticulocytosis at levels above 0.5 mg/m^3. Non-exposed controls did not have these findings. Stewart et al. (1945) study with pre-, during-, and post-TNT exposures in munitions workers showed a 9.6 to 14.2% hemoglobin drop with minimal reticulocytosis during exposure, but followed by an increasing reticulocytosis 48-h post-exposure; the air concentrations ranged between 0.3 and 1.3 mg/m^3. El Ghawabi and Ibrahim (1974) showed a 15% hemoglobin concentration drop in TNT-exposed munitions workers versus no change in a non-exposed control group; exposure levels of TNT for exposed group ranged from 0.2 to 1.0 mg/m^3.

Aside from anemia, TNT has been associated with other health effects to include hepatic necrosis, cataracts, dermatitis, and low sperm count. TNT is also mutagenic and considered to be a potential human carcinogen based on both human and animal studies (Bolt et al. 2006; Richter-Torres et al. 1995).

Systemic absorption of TNT in explosive workers is considered to be principally via the respiratory route necessitating respiratory protection once a time weighted average (TWA) standard is met (i.e., OSHA 8-h permissible exposure limit (PEL) -TWA of 1.5 mg/m^3 for private industry, ACGIH 8-h Threshold limit value (TLV) -TWA of 0.1 mg/m^3 for government industry, and the NIOSH 8 h Recommended exposure limit (REL)—TWA of 0.5 mg/m^3). NIOSH minimal respiratory protection guidelines for TNT, per the NIOSH Pocket Guide to Chemical Hazards (2005), recommend any supplied-air respirator with an Assigned Protection Factor (APF) equal to 10 for TNT air concentrations up to 5 mg/m^3. A major concern addressed by numerous authors, however, is the importance of the skin absorption route, which some have suggested is the most important route of TNT absorption in exposed workers (Richter-Torres et al. 1995; Sabbioni et al. 2005; Hathaway 1977; Hunter 1978). These skin absorption observations are corroborated by dermal absorption studies in animal models using radiolabeled TNT (Richter-Torres et al. 1995). Because of this skin absorption issue, NIOSH, OSHA, and the ACGIH all have a skin notation as part of their respective TWA standards. This suggests that (1) air monitoring alone may not be able to guarantee a healthful work environment, and that some sort of biological monitoring is required in addition; and (2) that respirator use alone may provide inadequate protection.

The following is a brief overview of TNT metabolism and excretion. TNT can be absorbed via respiration, ingestion, and through intact skin. It is rapidly and extensively metabolized as evidenced by at most only trace amounts of native substance (TNT) in the urine of humans and animals in some studies (Richter-Torres et al. 1995). Radiolabeled TNT in animal models has been shown to distribute to the blood, liver, kidney, spleen, lungs, brain, and skeletal muscle. One metabolic route is by the reduction of the TNT nitro groups to aminotoluenes followed by their oxidation to hydroxyarylamines via cytochrome P450. The other route is by the oxidation of the TNT methyl group to benzyl alcohol or benzoic acid. The last step involves benzyl alcohol and hydroxyarylamine conjugation with sulfuryl, glucuronide, and acetyl moieties. The major urine metabolites detected from this process are the aminotoluenes 4-aminodinitrotoluene (the major metabolite), 2-aminodinitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, and amino-nitrocresol. The aminotoluene metabolites will clear within 48 h of exposure cessation and usually impart a dark red discoloration to the urine (which may be confused with hematuria/hemoglobinuria unless a urinalysis rules this out) (Sabbioni et al. 2005; Richter-Torres et al. 1995; Channon and Mills 1944; Lemberg and Callaghan 1945; Bodeau 1993).

There continues to be a search for a biological marker that will correlate with exposure and clinical effects but this has not yet materialized; urinary metabolites clear quickly (within 48 h) and do not correspond well to end organ damage (Sabbioni et al. 2005; Bodeau 1993; Richter-Torres et al. 1995; author's experience). A promising venture by Sabbioni et al. (2005) looked at the relationship between hemoglobin adducts of sulfinic acid amides (formed when TNT metabolite nitrosoarene reacts with cysteine residues) and toxic effects. Although somewhat promising for cataract, splenomegaly, and hepatomegaly when controlled for confounders, it is doubtful whether this test will come to fruition as a standardized test for TNT effects (Sabbioni et al. 2005). In the United States, TNT biological monitoring takes the form of medical monitoring (no BEI for TNT currently per the ACGIH) and is accomplished by routine labwork to look for organ damage before that damage manifests clinically. The routine labwork for medical monitoring that was performed at the author's plant consisted of a complete blood count for anemia surveillance (CBC), liver function tests for hepatitis surveillance, lactate dehydrogenase (LDH), and urinalysis (UA) to support abnormalities of the CBC or LFTs (Bradley 2005). Also utilized was a handheld pulse oximeter to obtain an indirect measure of methemoglobinemia. Of the above medical monitoring, the earliest manifestation

of TNT-induced change involved pulse oximeter reading spO2 drops and hemoglobin/hematocrit drop in the CBC (Richter-Torres et al. 1995; author's experience). The red blood cell (RBC) indices usually remain normal; even when hemoglobin drops to the point of anemia, the indices were still usually within normal limits (i.e., normochromic, normocytic anemia) (Army 1976; Crawford 1954; Hathaway 1977; author's experience). The hemoglobin and hematocrit drops, according to Bodeau (1993), appear to be concentration dependent at TNT TWAs between 0.2 and 0.5 mg/m^3; this is supported by Gribova et al. (1983) who also demonstrated a concentration dependency, and by studies cited in the TNT ATSDR Profile (Richter-Torres et al. 1995). Nothing was mentioned as to whether or not the workers in these studies were in respiratory protection nor was there any mention of the hemoglobin drops being linear as a function of the TNT air concentrations.

The American Conference of Governmental Industrial Hygienists (ACGIH) adopted its current TNT TLV of 0.1 mg/m^3 in 1997 based principally on the German TNT Arbeitsplatzgrenzwerte of the same concentration established in 1988 (Deutsche Forschungsgemeinschaft (DFG) 1991) (Of note, as of 2008, the Arbeitsplatzgrenzwerte MAK value of 0.1 mg/m^3 has been abolished due to the reclassification of TNT in Germany as a class 2 carcinogen). The ACGIH authors cite the DFG: Occupational Toxicants studies on TNT (pp359–387), Gribova et al. (1983), and Hathaway (1977) stating that "altered liver parameters were seen in workers exposed to 0.3–0.8 mg/m^3, and changes in hematologic parameters were seen in workers exposed to 0.05–7.5 mg/m^3" (ACGIH 2001). Despite evidence going back years, OSHA continues to maintain a PEL of 1.5 mg/m^3. There is certainly substantial evidence for a lowering of the PEL to the present TLV level, this was in fact argued by Hathaway (1977) when he authored a review article arguing for a drop to the then TLV of 0.5 mg/m^3. Furthermore, according to Bodeau's (1993) review, he discovered that of 21 recorded TNT fatalities from aplastic anemia and toxic hepatitis during World War II that had air sampling data, only 1/3 of these were over 1.5 mg/m^3 (which in the 1940s was the TLV (MAC-TWA), and is still today the PEL). Usual industrial hygiene controls for TNT exposure may include process isolation, substitution (i.e., changing from TNT to another explosive, such as plastic bonded explosive (PBX)), education and training, local exhaust ventilation, administrative controls (such as job rotation, showering, plant provided garments, clean side/dirty side changing areas, etc.), and personal protective equipment (air-line respirator, or air purifying negative pressure with combination HEPA and organic vapor cartridges; also cotton coveralls, cotton caps, gloves, and conductive steel toe boots for static dissipation).

The literature review for this study was somewhat difficult as there was an inability to procure various primary sources, and therefore, reliance on some review articles became necessary. There is not a great deal of contemporary research being done on TNT presently outside of Sabbioni's group out of Switzerland; most of the primary literature goes back to the 1940s through 1970s time frame, as well as articles from the former eastern block and Soviet Union. Of note is that none of the studies involving TNT-induced anemia in workers involved workers using respiratory protection and neither does the ACGIH reference respirator use in any of the studies it utilized to justify their latest TLV. The skin notation evident for the TLV appears to be justified principally by (1) animal studies confirming a dermal absorption , and (2) by disproportionate quantities of urine metabolites at certain air concentrations that could not be explained by the respiratory route alone (ACGIH 2001; Woolen et al. 1986). Historically, the ACGIH adopted the skin notation for TNT back in 1961 based on animal models and worker observations, leading to the adoption of the notation by NIOSH and OSHA when these entities came into existence in the 1970s (OSHA adopted all of the 1969 TLVs as their PELs).

The objective of this present study is to corroborate the need for the skin notation by investigating if respiratory protected TNT workers are at risk for hemoglobin concentration drop and/or anemia development (the earliest systemic change secondary to TNT toxicity); no study thus encountered has looked at the skin absorption issue from the point of view of respiratory protected workers. The hypothesis propagated is that, based on the importance of the skin absorption route, respirator use alone is insufficient to provide protection against hemoglobin concentration drop and/or anemia cases in workers working around open molten TNT.

The target population is respirator protected TNT munitions workers who work with open molten TNT who may be at risk for hemoglobin drop and/or anemia development via the TNT skin absorption route.

Materials and methods

A retrospective observational study was chosen where TNT exposed workers served as their own controls; no independent control group was used. The data had been procured prospectively by repeated surveys when the investigator ran the TNT medical monitoring program at an ammunition plant; the specific time frame retrospectively reviewed was between October 2006 and April 2007. The de-identified data included worker baseline hemoglobin concentrations, follow-up hemoglobin concentrations during TNT exposure, and breathing zone and/or general area

Table 1 Number of workers in each group with shift, operation, and type of respiratory protection

Group	Total workers	Male workers	Female workers	Shift	Operation	Respiratory protection
1	15	11	4	Day	Demilitarization	APR[a]
2	16	12	4	Night	Demilitarization	APR
3	12	12	0	Day	Loading	Air-line[b]
4	16	12	4	Day	Demilitarization	APR
5	18	17	1	Day	Demilitarization	APR
6	14	14	0	Night	Demilitarization	APR
7	13	12	1	Day	Demilitarization	APR
8	19	14	5	Day	Loading	Air-line

[a] APR: Full face 3 M or North negative pressure air purifying respirator with combination HEPA and organic vapor cartridges

[b] Air-line: Positive pressure air-line respirator (hood)

air sampling for TNT vapor done as close in time as possible to the follow-up hemoglobin labwork.

The convenience samples consisted of 6 groups of respiratory protected (North or 3 M full face-negative pressure air purifying respirators supplied with combination organic vapor and HEPA filter cartridges) munitions demilitarization workers who worked in a TNT melt-out facility, who were exposed to TNT vapor, and were at risk for TNT skin absorption between October 2006 and April 2007 (2 of these 6 groups were night shift); and an additional two groups from another facility involved in a bomb loading operation that were respiratory protected (positive pressure air-line), but at risk for TNT skin absorption in October 2006 and February 2007. These workers' additional PPE ensembles consisted of rubber gloves, conductive steel toe boots, flame retardant cotton coveralls and cotton caps. This PPE garb is intended to stave off any potential for detonation due to static build-up, but provides no protection from TNT vapor skin contact. The workers' caps, coveralls and work undergarments were provided and laundered by the plant. The respiratory protection program followed the tenets of 29CFR1910.134 and included medical clearances, annual quantitative fit testing, worker training, respirator storage/cleaning/maintenance, cartridge change-outs, etc.

Data analysis was performed using SPSS version 17.0 for Windows. Eight data sets were generated corresponding to the aforementioned 8 groups of workers where, for each group, mean hemoglobin level changes were calculated using the individual workers pre-exposure baselines and during-exposure follow-ups. A two-tailed paired t test was used for each group of workers to see which group(s) had a statistically significant mean hemoglobin concentration change during TNT exposure; 95% confidence intervals were also determined. Hemoglobin concentration is normally distributed in the population, and for purposes here, the null hypothesis is no mean hemoglobin concentration change, with the alternative being during-exposure mean hemoglobin concentration change compared with pre-exposure baseline. These t-tests were conducted at an alpha level of 0.05. Since group hemoglobin change observations over time was not a goal in this study, and because worker complement changed within groups, a repeated measures ANOVA was not incorporated in the analysis. However, a curve estimation analysis was performed to examine best fit regression models for the relationship between TNT air concentration and hemoglobin change.

The 8 groups varied in size from 12 to 19 workers consisting predominantly of men with between 0 and 5 women per group (Table 1). Baseline pre-exposure labwork, per standing operating procedure were all less than 1-year old. Follow-up during exposure labwork would ideally have been conducted post-shift, post-workweek but was usually collected Mondays post-shift. This was not detrimental as anemia recovery times usually ranged from about 2 weeks to 60 days per our experience. All complete blood counts (CBCs) were sent to the clinic's Joint Commission accredited contract laboratory in the town's regional medical center for processing.

Corresponding to the mean hemoglobin changes calculated previously, all breathing zone (BZ) and general area (GA) TNT vapor concentration air sampling values were obtained closest in time to each groups' follow-up during-exposure labwork. Numbers of samplings varied between each group and ranged between 2 and 8 samples each. The industrial hygiene section utilized the OSHA TNT air sampling organic method #44 (OSHA, 1983) and calculated out a 10-h TWA per sample (the workweek was 4 days, 10 h shifts). The samples were taken over the full duration of the shifts and were monitored by an on site industrial hygiene technician. Most of the BZs taken involved the pump being placed on more than one worker as the workers rotated through the facilities' workstations. Job rotation was implemented as the main administrative control, but BZ workstation evaluations were ongoing not only as a means of monitoring worker exposure but also to

gage ongoing attempted engineering fixes to reduce the TNT vapor concentrations about the time frame of this study. The plant, by regulation, fell under the TNT TLV-TWA of 0.1 mg/m^3 (or 0.08 mg/m^3 for the 10 h plant shifts) even though most of the time, the air sampling was below the present OSHA PEL-TWA of 1.5 mg/m^3 (or 1.2 mg/m^3 for 10 h). The samples were analyzed at the plant's laboratory using OSHA method #44 where samples were desorbed with acetone and analyzed by gas chromatography using a thermal energy analyzer (TEA) with an explosives analysis package (EAP). Assuming normal distribution of TNT vapor, means for each of the 8 groups were calculated for exposure assessments and regression modeling purposes. Medians were also calculated as true distribution is likely to be lognormal due to sampling selection methodology (Perkins 1997).

Clinical significance in hemoglobin concentration reduction was defined as any drop in hemoglobin that fell below the lower laboratory limit of normal (anemia: <12.2 g/dL for women and <14.1 g/dL for men) or a hemoglobin drop of greater than 2 g/dL within 1 month within the reference laboratory's normal reference range.

Since study design was observational, the following were the default selection inclusion criteria: Healthy workers not controlled for age (older employees given counseling on their increased risk of TNT-induced anemia), sex, ethnicity, smoking status, alcohol use (employees who used alcohol cautioned about TNT's and alcohol's association with hepatitis), SES, BMI, duration of time in TNT facility (including carry-over from one follow-up to another. Duration of time only controlled by clinic when worker(s) was pulled from open TNT work due to the above criteria of becoming anemic, or having a hemoglobin drop of greater than 2 g/dL within the normal range), and duration of time at particular work station due to job rotation but controlled for respiratory exposure to TNT vapor via respiratory protection. Due to subjects serving as their own controls, most demographics were not collected.

By the plant's TNT biological monitoring SOP, selection exclusion criteria included the following: Pre-existing anemia of any cause (if menses a cause, must have normal hemoglobin/hematocrit off cycle), pre-existing liver disease and/or elevated liver function tests (LFTs), Glucose-6-phosphate dehydrogenase (G6PD) deficiency, any medications such as isoniazid, phenylbutazone, phenytoin, methotrexate, and others that have the potential to cause marrow suppression, cholestasis, or hepatocellular necrosis, etc. (permitted those with daily acetominophen use or HMG-CoA reductase inhibitor use if LFTs were within normal limits), any medical conditions that would predispose to anemia of chronic disease (i.e., Hepatitis, poorly controlled diabetes mellitus, etc.), any conditions that may worsen with induced anemia (COPD, ASCAD, ASPVD, CHF, etc.), pregnancy (left choice to woman, by SOP recommendation is to stay out; clinic-educated woman on dangers to fetus of TNT exposure), any TNT-induced anemics who have not yet recovered after removal, and any condition that would be disqualifying for negative pressure air purifying respirator use.

The following is a step-by-step outline of the munitions demilitarization/TNT reclamation process in the facility where the aforementioned 6 groups of workers were working: (1) Fork truck moves pallet of bombs from boxcar to holding area where de-palletization occurs. (2) Fork truck lifts bombs onto breakdown/roll table where base plate and cone tip are removed and bomb body is wiped down. (3) Temporary base plate is installed to accommodate scissor hoist. (4) Bomb rolled on table to steam out operation of fuse well (hot steam emitted via a cone that fits over fuse well area in order to loosen the tar binding, well is then removed via specialized tool and placed into scrap bin). (5) Bomb is then rolled to tilt table. (6) Tilt table takes bomb from horizontal position to vertical position nose down. (7) Scissor hoist is then placed around temporary baseplate. (8) Bomb is then hoisted 3 levels up to copula area where the autoclaving operation takes place. (9) Bomb is placed into autoclave and sealed; it will cook for around 4 h at a temperature range of 180–200 degrees Fahrenheit (melting temperature of TNT). (10) Melted out TNT exits the nose cone area through the fuse well opening and is conduced via gravity through an insulated enclosed trough system to a kettle with hot holding temperature of 180F. (11) Molten TNT then exits kettle through an enclosed trough in a controlled fashion onto an enclosed extruder belt, which is water cooled from below. (12) Along the extruder belt length, the molten TNT is cooled until it is re-solidified as flake by the belt's end. (13) TNT flake is then collected in plastic bags within cardboard boxes in an enclosed flake room at the extruder belt's end; when a box is full, it is conveyed out of the flake room via a belt system to the weighing and QA stations. (14) Box is then sealed, labeled, and palletized. (15) Meanwhile, back up in the autoclave area above: Empty bomb body is hoisted out of the autoclave and lowered back down to tilt table where scissor hoist and temporary base plate is removed (temporary base plate is recycled back to the breakdown table). (16) Bomb is then re-oriented from the vertical to the horizontal in the tilt table. (17) Bomb body is then rolled out of tilt table onto roll table where the explosive well of the bomb body is scraped out to remove remnants of the tar lining (tar lining provides insulation between the metal portion of the bomb body and the explosive charge). (18) When bomb body is certified TNT and tar free, it is moved by fork truck to grade 1 scrap holding area outside the building. (19) Reclaimed palletized TNT is trucked out of facility to an indoor storage

area; and fuse wells and bomb bodies are trucked to an outside scrap storage area.

The major source of TNT vapor exposure within the demilitarization facility was two open areas of the trough gallery on the mezzanine level between the ground level and the copula where the autoclaves were right about in the center of the building. The aforementioned step-by-step process was designed to be a closed process with an apparently robust exhaust ventilation system but one problem evidently had not been foreseen. The problem involved the unanticipated melting out of some of the bomb bodies' tar lining along with the TNT. The molten tar lining contaminates the reclaimed TNT and hence is a quality assurance (QA) issue, and it also tends to clog up the trough and kettle systems. Another less important source of TNT vapor was a removed panel from the side of the enclosed extruder belt housing that allowed for the escape of water vapor (as well as TNT vapor); the water vapor from the belt cooling system would contaminate the TNT (there is a QA standard for what an acceptable amount of water content is permitted in the product—i.e., TNT purity). Some grab samples in the building revealed a TNT vapor concentration gradient from these two sources outward (these two sources were very close to one another, one being on the mezzanine and the other almost directly below on the ground level). Also, there were some fugitive emissions from the opening of the autoclaves when spent bomb bodies were removed (2–3 times per day for maybe half an hour). Due to the tar problem, there is a necessity for a mezzanine work station where workers called tar dippers ladle out clumps of tar from the trough system at a point just under the autoclaves but before the kettle; this is the reason for the breach in the enclosed trough gallery system mentioned previously. Part of the job rotation objectives alluded to earlier is to relieve the tar dippers as the mezzanine level appears to be the point of maximum TNT exposure. During the present biosurveillance and air sampling run, various engineering solutions for these problems were being attempted in order to eliminate the tar dipper positions, re-establish the integrity of the closed system, and target air sampling levels to the TWA-TLV.

The operation's worker numbers and work stations are as follows: 1 supervisor, 1 fork lift operator, 2–3 breakdown table, 2–3 fuse well steam out, 2–3 bomb scraping, 1 extruder belt/kettle operator, 1–2 autoclave operators, 2–3 tar dippers, 1 flake room worker, 1 weigher/palletizer, 1 QA inspector.

Results

There were no statistically significant mean hemoglobin changes at the following mean TNT air concentration values: 0.12, 0.23, and 0.27 mg/m^3 (median values, respectively, 0.07, 0.16, and 0.10 mg/m^3). Also, no anemia cases were evident at these levels. There were, however, statistically significant mean hemoglobin level drops and anemia cases at the following mean TNT air concentration values: 0.31, 0.47 mg/m^3($\times$2), 0.7, and 1.31 mg/m^3 (median values, respectively, 0.27, 0.47 mg/m^3($\times$2), 0.40, and 1.24 mg/m^3). The number of munitions workers per group tested composed the total complement of workers on shift the day of testing. Of the 15 total anemia cases, 2 were woman (non-menstruating) (Table 2).

Curve estimate analysis revealed both exponential and inverse non-linear regression models as best fits for the observed data with both p values <0.05. The inverse model (lowest of the two p values) is presented here (Table 3; Fig. 1).

$$y = b0 + (b1/x)$$

Discussion

Utilizing the TNT PEL (1.2 mg/m^3), REL (0.4 mg/m^3), and TLV (0.08 mg/m^3) 10-h TWAs as benchmarks, the results of this study show that mean and median TNT air concentrations about the TLV were protective against anemia and statistically significant hemoglobin changes in those respiratory protected workers who are at risk for anemia per the TNT skin absorption route (ref. to first 3 groups above in "Results" section). A question does remain, however, as to what the anemia risk might be for those workers who work below the TLV and, hence, do not 'require' respiratory protection. With these first 3 groups, it is unlikely that these workers are not absorbing any TNT at all; they are more than likely receiving inconsequential doses with regard to anemia formation (i.e., a burden that the body can handle effectively). Two of these three groups were in full face negative pressure air purifying respirators, so there was the possibility of breakthrough. However, even if some break through occurred, it evidently did not affect these 2 groups. The other group was in positive pressure air-lines that revealed no breakthrough on under hood BZ sampling (this sampling was not part of the present study, but was conducted shortly after the air-line system was installed in the loading facility).

The results obtained from the last 5 groups of workers above appear to confirm the necessity for the skin notation for the PEL and REL particularly. Mean and median TNT air concentrations about the REL and PEL were not protective against anemia or statistically significant hemoglobin change (reduction) in those workers who were respiratory protected but at risk for anemia per the TNT skin absorption route.

Table 2 Mean hemoglobin change as a function of mean TNT air concentrations; hemoglobin change analyzed via paired *t*-tests at an alpha of 0.05, and with 95% CIs

Mean/median TNT air concs (mg/m^3) (anemia cases)	Number of air samples taken per group	Number of munitions workers per group	Mean Hgb Chg (g/dL)	*p* value (alpha 0.05) 2-tail/1-tail	95% CIs
0.12/0.07 (0)	8	15 (group 1)	−0.280	0.189/0.0945	(−0.174, 0.154)
0.23/0.16 (0)	5	16 (group 2)	0.125	0.489/0.245	(−0.258, 0.510)
0.27/0.10 (0)	5	12 (group 3)	0.358	0.090/0.045	(−0.065, 0.782)
0.31/0.27 (4 male)	5	16 (group 4)	0.594	0.001/0.0005	(0.277, 0.910)
0.47/0.47 (3 male)	2	18 (group 5)	0.478	0.021/0.0105	(0.081, 0.875)
0.47/0.47 (1 male)	2	14 (group 6)	0.493	0.002/0.001	(0.215, 0.771)
0.70/0.40 (4 male)	5	13 (group 7)	0.577	0.006/0.003	(0.200, 0.950)
1.31/1.24 (1 male, 2 female)	4	19 (group 8)	0.490	0.029/0.0145	(0.056, 0.923)

Table 3 Inverse non-linear regression model summary and parameters

Equation	Model summary					Parameter estimates	
	R square	*F*	*df*1	*df*2	Sig.	*b*0	*b*1
1/tntair	.845	32.751	1	6	.001	.729	−.115

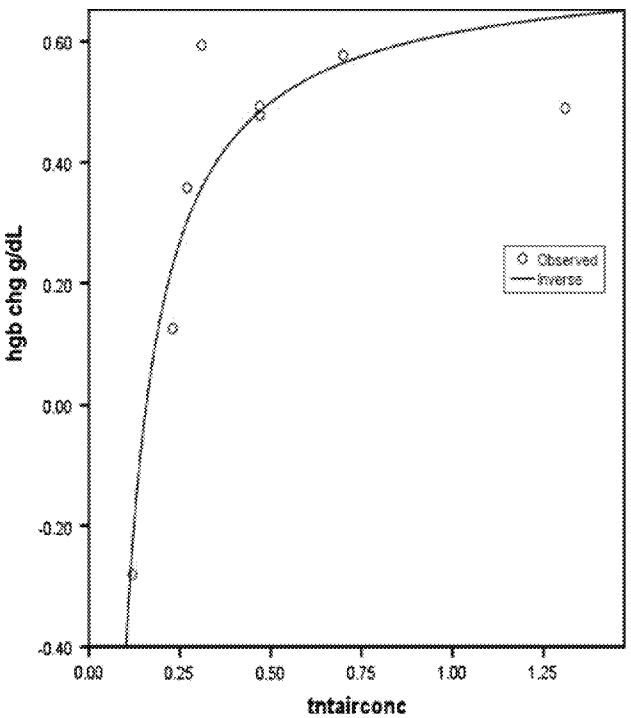

Fig. 1 Inverse non-linear regression model showing hemoglobin change (g/dL) as a function of TNT air concentrations (mg/m^3)

This is the first known study, that the investigator is aware of, that looked at anemia risk and statistically significant hemoglobin concentration change in respiratory protected TNT munitions workers. The assumption is that the dermal route is the main TNT absorption danger for these workers; negative pressure respirator breakthrough and ingestion routes were probably minimal. In evaluating the dermal absorption problem in the past at the plant, the industrial hygiene section confirmed that the cotton coveralls provided no barrier to TNT vapor contacting the skin. Although intuitive that TNT vapor was in contact with skin, confirmation was done by using Webster's reagent on the skin of munitions workers post-shift in a non-TNT setting. Webster's reagent is an old colorimetric indicator of TNT presence that was developed in the 1920s; on contact with TNT, it changes to a dark red color. This testing, along with literature precedents of dermal TNT absorption and TNT-induced anemia in man and in animal modeling (see Introduction), plus similar observations at the plant that mirrored this study, revealed that it is more likely than not that TNT-induced hemoglobin reduction and the development of anemia cases came about through the skin absorption of TNT. An attempt at dermal absorption control that was tried at the plant was the use of Tyvek suits. This was infeasible due to lack of heat dissipation in an already hot environment, and more dangerously, due to the generation of static electricity.

Part of the plant's standing operating procedure (SOP) on TNT medical monitoring provided for the kind of analysis done here in this study. It became apparent early on that anemia cases (plant-wide in all open TNT operations) were random, as there did not appear to be any correlations to duration of time in building, personal BZ air monitoring levels, repeat cases, particular work stations, time spent at particular work stations, personal hygiene, sex, ethnicity, medications used, etc. as to whom and when anemia would become evident (previous investigation in 2004). As an aside, even though age was not predictable as to who became anemic, the experience has been that the older workers with anemia took longer to recover than the younger workers. Another study at the plant looked into the above factors again and even included a TNT urine

metabolite, 4-aminodinitrotoluene (ADNT) (non-standardized test). This test showed no correlation with anemia cases, duration of time in TNT building, particular work stations, duration of time at a particular work station, age, or sex. In fact, there were only nine 4-aminodinitrotoluene positives in 112 post-shift TNT worker samples taken; and in only one of these nine positives, did a worker have anemia (out of a total of 23 anemia cases from the 112 tested). The possibility of laboratory error though could not be ruled out, as this test has never been done on a routine basis (unpublished data). Anemia cases at the plant could appear after just 1 or 2 days post exposure, after a few months, or not at all. With the anemia cases in this present study, there were no repeats; every anemia case was a different worker. Whether this was due to job rotation, lack of respirator compliance (or large breakthroughs) in those who became anemic, or individual susceptibilities with regard to marrow response timing and intensity, is unknown. If there were repeaters (there were some repeaters at the plant overall, but no repeaters in this study), in general, it may be easier to discover a problem (i.e., lack of hygiene, broken respirator, medication use, missed medical history, etc.). For the reasons stated above, it was decided to look at whole groups of workers in the TNT environments at monthly cross-sections with TNT air concentrations and associated hemoglobin changes to see what might be going on with the workers as a cohort (see materials and methods section). The questions then became: (1) Are statistically significant mean hemoglobin changes predictive of the development of anemia cases, and (2) At what mean TNT air concentrations are these phenomena occurring? This method of surveillance seemed to fit well with newer plant administrative policies of job rotation, attempted engineering controls, enhanced personal hygiene emphasis, and providing and laundering all work clothes (under garments included). The administrative control of worker rotation involved rotations in and out of the TNT buildings, and rotations in and out of higher exposure areas within the TNT buildings. Management took the initiative to implement this secondary to the air sampling data as an interim control until an engineering fix could be found even though our hemoglobin data revealed no conclusive patterns of susceptibility. Any anemia cases would be removed from exposure and followed up in clinic, and results of the statistical analyses and the number of anemia cases would be shared with IH, engineering, management, and the safety office.

When during-exposure labwork indicated the onset of anemia, the worker was pulled from open TNT, and a clinical evaluation took place looking for alternative causes to explain the anemia (i.e., menses, blood donation, new disease status, such as pernicious anemia, new meds, etc.). Symptoms were addressed (fatigue, tiredness, orthostasis, headaches, etc.), as well as problems related to PPE, rotation schedule, and personal hygiene. A physical examination took place focusing on vital signs, spO2, presence of splenomegaly, pallor, and cyanosis; the lack of physical findings was usually the rule. Out of the many anemia cases pulled over the years the investigator was at the plant, the only effect modifiers recalled were menses and blood donation. The clinic always considered anemia as caused by TNT unless proven otherwise. One case of pernicious anemia did develop in a worker who, once treated with B12 and recovered, was permitted back into the TNT environment (not in present study). All cases of TNT-induced anemia resolved once the worker was removed from exposure; the recovery time usually took from 2 weeks to 60 days, at which time they were permitted re-entry into the TNT buildings (most desired a return to duty; but others did not want to go back into the environment, their decisions were supported by the clinic and the plant, and the plant usually found work for them in alternative operations). The anemia cases over the years the investigator was at the plant were best characterized as mild hemolytic anemia. The most notable finding was a normocytic, normochromic anemia, which was occasionally supported by reticulocytosis, low haptoglobin, high AST, and/or high LDH levels (although these latter two could certainly have been influenced by TNT's hepatotoxicity). Conspicuously absent were bite cells, Heinz bodies, spherocytes, and schistocytes, however. As with this study, anemia cases were usually mild with no worker usually surpassing a 10% reduction in hemoglobin concentration.

Shortcomings of this study include less than robust TNT air sampling sample numbers per group. Statistical sampling would have been desirable, but was very cost prohibitive. For this reason, both the mean and median were used in this study to cover normality and lognormality. Interestingly, both values per group were relatively close to one another when measured against the PEL, REL, and TLV benchmarks; however, the group 3 mean appears to have been overly influenced by the 1.1 mg/m^3 outlier. Another shortcoming may be the lack of use of NIOSH recommended air-line respirators for open TNT work in the 6 groups working in the demil facility. These workers wore NIOSH approved 3 M or North negative pressure air purifying respirators supplied with organic vapor and HEPA filter combination cartridges instead (i.e., NIOSH approved 60921 organic vapor cartridge and P100 (HEPA) particulate filter). As of this writing, the demil facility does now have air-line respirators in place. This shortcoming is likely not detrimental, as the use of appropriate NIOSH approved combination cartridges, NIOSH approved full face respirators, and the clinic's very robust respiratory protection program, should put the APF at 50 (5 orders of

magnitude greater than the NIOSH recommended supplied air respirator of at least APF 10 for TNT levels up to 5 mg/m^3 8 h TWA) (29CFR1910.134).

Per the inclusion criteria, due to the observational nature of the study, workers were not controlled for time exposed to open TNT. Rotations on and off the operation, as well as rotations within the operation took place regularly. Also, those who became anemic were pulled out of the operation by plant medical personnel thus taking away from the worker complement of the operation. There were workers who remained for more than one round of sampling, and there were workers put into the operation maybe 2–3 days before a follow-up during-exposure hemoglobin level was to be drawn. This process continued throughout the 8 sampling periods. Conceivably, there could be carry-over problems (bias away from null) from workers staying in for 1 or 2 rounds of follow-up during-exposure sampling. Conversely, minimal exposure for those who may have entered the cycle only a couple of days before a sample was to be taken could conceivably bias the group toward the null. But, as stated above, the previous experience with looking at exposure duration times and anemia development revealed no correlation; this lack of correlation is supported by Gribova et al. (1983) and Army (1976). Therefore, the issue with job rotation may not be detrimental to this study. In fact, the rotations were business as usual, so from an observational standpoint, for the convenience samples used here, internal validity was met. External validity would certainly be based on operational, PPE, administrative controls, and engineering controls similarities to our samples, so generalizability may, or may not be appropriate depending on the circumstances. Also, no attempt was made in the analysis portion to control for carry-over.

The cause for no correlation between exposure time and anemia development could most likely be due to individual differences in bone marrow response timing and intensity (and as a caveat, to the timing of the medical monitoring labwork with regard to where a particular worker might be in his/her marrow response when the blood is drawn). To best judge what may be going on overall with regard to differing individual susceptibilities and responses in otherwise healthy workers (per exclusion criteria) would be to look at what is going on with all workers in a particular single building operation, such as was done here. It may be that at those lower TNT air concentrations, most workers can mount a sufficient marrow response that does not generate a statistically significant mean hemoglobin concentration drop for the whole group. This was especially pronounced at the lowest mean TNT level in the study (group 1) where the group was actually able to generate a mean hemoglobin concentration that was greater than their pre-exposure baselines (although not a statistically significant increase), even though there was no different process in place as far as job rotations in and out of the building, and job rotation work station to work station within the building. The finding with this group, as relates to the TNT air concentration level, was probably the natural dilution ventilation that was maintained throughout 2–3 weeks during a warm spell in October of 2006 rendering a mean TNT 10-h TWA of 0.12 mg/m^3 (median 0.07 mg/m^3) in the building. The converse is probably true at higher TNT air concentrations where some workers cannot mount an effective marrow response, or their response time is longer in coming, compared to other workers. Since bone marrow biopsies are out of the question and reticulocytosis rare until after removal from TNT (Army 1976; Stewart 1945), the results of this study at least show that as statistically significant mean hemoglobin drops become apparent, it may be time to expect some anemia cases.

The plotted data and inverse non-linear regression modeling reveals a curvilinear dose–response curve with regard to mean hemoglobin change as a function of mean TNT air concentrations. This is not inconsistent with the dose–response findings described in Gribova et al. 1983; Army 1976; Bodeau 1993; and El Ghawabi 1974. However, caution should be exercised by not extrapolating beyond the observed values range, and by considering possible skew of the TNT air sampling distribution. This model is inflexible, as no matter how great the TNT air concentration, the corresponding hemoglobin drop can never exceed 0.729 g/dL. Nor can hemoglobin increase ad infinitum as TNT air concentrations approach zero. The flattening of the curve at higher TNT values in the model is likely due to group bone marrow response to replace damaged erythrocytes, with the majority of workers having a marrow response that levels out their hemoglobin change but does not necessarily resolve it back to pre-exposure baseline. It may be that there is a step-wise phenomenon playing out with the marrow able to respond to increasing RBC destruction by leveling off at certain TNT doses; and as the dose increases, it is followed by another drop followed by another leveling, and so forth, until aplasia occurs at some critical TNT load and leveling or recovery are not possible. Of note, the three lowest TNT values, and concomitant hemoglobin difference levels, were all independent, as they were taken from demil dayshift, demil night shift, and load dayshift, respectively.

This study does suggest that, despite likely individual worker bone marrow responses with resistance or susceptibility to anemia at given TNT doses, the average ambient TNT air concentration appears predictive as to the development of anemia cases in worker groups in respiratory protection (at least in the groups studied here; may not be entirely generalizable to other operations). Which particular individual worker(s) within the group(s) actually

become anemic is another matter. The mean and/or median ambient TNT air concentrations could be used to evaluate engineering controls to reduce the mean/median to levels close to the TLV, provided worker rotation is part of the exposure reduction plan as well.

Alternative causes to explain the anemia cases and hemoglobin drops in the groups besides TNT exposure were considered. Normal fluctuation in healthy persons' hemoglobin levels, which would be quite narrow over the time period studied here, were considered, but due to sufficient a priori evidence of the association between TNT and anemia and hemoglobin drop (and anemia cases considered as occupational sentinel health events in TNT workers in the US), this natural fluctuation was taken to be only a small effect modifier without much significance (i.e., results not likely due to chance). Furthermore, the conditions of work for these munitions workers may be more conducive to hemoconcentration rather than anemia if they were not working with open TNT. Factors such as negative pressure respirator wear leading to a more labored breathing with possible small oxygen desaturations, and manual labor necessitating increased oxygen demand may be factors in creating a relative polycythemia. Dehydration from the hot environment could contribute to hemoconcentration as well. In order to better characterize these factors, an independent control group would have been necessary; unfortunately this option was not available for this study.

Standard morbidity ratios (SMRs) were not calculated for anemia in these workers over anemia in the general population. The Healthy Worker Effect (HWE) bias most certainly would have the general population with a higher proportion of anemics due to old age considerations. An age stratification was not attempted here. Furthermore, the anemia cases in this study all resolved back to their pre-exposure non-anemic baselines once they were removed from open TNT for a sufficient amount of time (i.e., no stable prevalence).

As a last point in helping further substantiate the TNT skin absorption route as important, is that there appeared to be no real difference in protection from anemia cases and mean hemoglobin drops between the 2 groups in air-line respirators and the 6 groups wearing the negative pressure air purifying respirators.

This study adds to the body of knowledge already in existence that there should be a consideration of a new TNT OSHA PEL based on the present ACGIH TLV of 0.1 mg/m^3 (8 h TWA) in the United States.

Lastly, because the German MAK served as the principle source used by the ACGIH in establishing their present TNT TLV, a re-evaluation of the TLV should be considered due to the 2008 reclassification of TNT as a human carcinogen in Germany and termination of the MAK value.

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